The association of the slow acetylator phenotype with bladder cancer

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SUMMARY There is an association between exposure to aromatic amines and the development of bladder cancer. Aromatic amines such as are known to occur in tobacco smoke are polymorphically acetylated. One hundred bladder cancer patients have been acetylator phenotyped. Only three of them were non-smokers at the time of diagnosis. This new series, together with four previous series (each with its own control), have been statistically analysed together.

The result shows a significant association between the slow acetylator phenotype and bladder cancer. The slow acetylator phenotype is associated about 39% more with bladder cancer than is the rapid acetylator phenotype. This association can be interpreted in one of two ways: (1) rapid acetylators may be protected against developing bladder cancer because they are better able to render amines non-carcinogenic by acetylation, or (2) slow acetylators have greater survival with bladder cancer than rapid acetylators. Further evidence will be required to differentiate between these alternatives.

A key biological puzzle is to understand how a known environmental agent reacts with a genetic polymorphism in such a manner that some subjects of a given phenotype are more prone to develop a particular disorder. Bladder cancers are known to occur at a higher rate in subjects exposed to aromatic amines either in tobacco smoke or in their occupations.¹

It has been found in both human and rabbit populations that some carcinogenic aromatic amines (for example, amino-fluorene, benzidine, and 2-naphthyl-amine) are polymorphically acetylated by the same N-acetyl-transferase that is responsible for the polymorphic acetylation of a number of drugs.^{2,3} In this genetic polymorphism slow acetylation is an autosomal recessive phenotype.⁴ These considerations have led to the suggestion that the two human acetylator phenotypes may differ in their predisposition to develop neoplastic disorders believed to be caused by aromatic amines. This idea is based on an analogy with the now established fact that genetic constitution can predispose to drug toxicity.⁵ An obvious example to test the hypothesis is bladder cancer.

Three previous publications⁶⁻⁸ give information on acetylator phenotypes of bladder cancer patients. More information is needed to reach a valid conclusion. This article reports the acetylator phenotypes

FIGURE The results of acetylator phenotyping 100 patients with bladder cancer. Rapid acetylators are defined as having an ordinate value >40 and an abscissa value >70 , whereas slow acetylators have values below these limits.

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TABLE 1 Bladder cancer patients subdivided by clinical criterion and acetylator phenotype.

Clinical criterion	Slow acetylators	Rapid acetylators	χ^2_1
Age at diagnosis			
< 50	6	2	With Yates 0.029
50 and over	60	32	
Sex			
Male	45	29	With Yates 2.58
Female	21	5	
Smoking			
> 20 cigs/day	29*	15	0.057
< 20 cigs/day	31	17	
Industrial exposure			
Has occurred	11†	8	0.943
Has not occurred	44	19	
Bladder cancer‡			
Grade 1	40	20	0.16
Grades 2, 3, 4	20	12	

For each row $p > 0.10$.

*In addition to the 92 cigarette smokers in the table there were also 5 pipe smokers and 3 non-smokers.

†In addition to the 19 employed in the chemical, dye, and rubber industries, there were another 18 whose exposure status was uncertain (exposure to paint solvents and engine oils and domicile next to chemicals factory).

‡No histology available on 6 patients; one had squamous metaplasia and another transitional cell papilloma on histology but diagnosed as cancer of the bladder on other evidence.

of a series of bladder cancer patients and analyses all the available data.

Patients

Patients treated for bladder cancer were obtained from a register held in the Regional Urology Unit at the Royal Liverpool Hospital. These patients are admitted for periodic cytoscopic examination.

They were non-random in several ways. They were survivors whose dates of birth varied from 1903 to 1953. Their year of diagnosis varied from 1964 to 1981. Their age at diagnosis varied from 23 to 73 years. They were further selected because they lived within 10 miles of this Department and because they agreed to participate in this experiment. All were tested between April and September 1982. Their phenotyping test was carried out at their home. All patients and their family doctors gave informed consent for the phenotyping procedure to be carried out.

Materials and methods

The phenotyping procedure used was method II of Evans⁹ which involves giving one oral dose of sulphadimidine (syn: sulfamethazine) and taking one blood and one urine specimen for analysis. The laboratory analytical procedure was carried out as described by Eze and Evans.¹⁰

Results

One hundred patients were phenotyped (figure). The pattern of the percentage acetylation of sulphadimidine in plasma and urine closely resembled that published for normal subjects (see, for example, Viznerova *et al*¹¹).

Sixty-six patients were slow acetylators giving as the frequency of the recessive allele $q = 0.81$ se (q) = 0.03 (see Karim *et al*¹² for comparative data). The mean age at diagnosis was not significantly different

TABLE 2 Test of association between bladder cancer and acetylator phenotype. (Modification by Haldane¹⁵ of the method of Woolf¹⁴)

Reference	No of subjects				Approximate relative risk x	$\log_e x$ $= y$	Sampling variance V	Weight $\frac{1}{V} = w$	Significance of difference from zero wy^2
	Bladder cancer		Controls						
	Slow	Rapid	Slow	Rapid					
Lower <i>et al</i> ⁶ Danish*	46	25	38	36	1.7288	0.5474	0.1124	8.8963	2.6657
Lower <i>et al</i> ⁶ Swedish*	80	35	79	39	1.1267	0.1193	0.0776	12.8827	0.1834
Cartwright <i>et al</i> ^{8†}	74	37	118	89	1.4921	0.4002	0.0592	16.9016	2.7054
Woodhouse <i>et al</i> ^{7‡}	21	9	16	13	1.8517	0.6161	0.2757	3.6270	1.3767
Present series	66	34	510§	342	1.2932	0.2571	0.0484	10.6748	1.3666

Weighted mean value of $y = Y = \frac{\sum wy}{\sum w} = \frac{20.7208}{62.9824} = 0.3290$.

SE of $Y = (\sum w)^{-\frac{1}{2}} = 0.1260$.

95% fiducial limits of $Y = Y \pm t_{0.05} (\sum w)^{-\frac{1}{2}} = Y \pm (2.78 \times 0.126) = 0.6793$ and -0.0213 .

Antilog of $Y = X$ = combined estimate of values of $x = 1.389$.

The equivalent X values to the 95% fiducial limits of Y are 1.9725 and 0.9789.

Significance of difference of X from unity = $\chi^2_1 = \frac{(\sum wy)^2}{\sum w} = 6.8170$ ($p < 0.01$).

Heterogeneity is tested by $\chi^2_{N-1} = \sum wy^2 - \frac{(\sum wy)^2}{\sum w} = 1.4824$ ($p > 0.10$).

*Technique of phenotyping: Weber and Brenner.²⁶

†Technique of phenotyping: monoacetyl dapsone/dapsone ratio in plasma.

‡Technique of phenotyping: plasma isoniazid half life.

§Pooled Liverpool control data.

in the two phenotypes (57.3 years in the slow and 58.6 in the rapid).

These patients have been sub-divided in relation to various characteristics (sex, age, smoking, history of industrial exposure, and histological type of bladder neoplasm) (table 1). In no case was there any hint of association with the acetylator polymorphism. At the time of diagnosis only three patients were non-smokers. There are no satisfactory control data available on the relationship between smoking habits and the acetylator polymorphism. Several retrospective studies have indicated that cigarette smokers have approximately twice the risk of non-smokers of cancer of the bladder.¹³ Positive industrial exposure was principally in the chemical and rubber industries where amines are known to have been employed. The 19 persons deemed to have been 'exposed' had worked in the rubber and chemical industries for varying periods most for many years. Two had worked in these industries for less than 7 years, one for 5 months, and one for 12 months.

The present results, together with four previously available series, have been examined by the method of Woolf¹⁴ as modified by Haldane¹⁵ (table 2).

Suitable control information for the present bladder cancer patients has been obtained by pooling data which have previously been published on several series of Liverpool white British subjects.⁹ (series I)^{10 16-25} There is a highly significant association between bladder cancer and the slow acetylator phenotype and there is no heterogeneity between the results from different series.

Discussion

The information shown in the figure closely resembles the results obtained when populations of healthy subjects have been phenotyped using the same technique, which is evidence indicating that any residual activity of bladder cancer did not interfere with the phenotyping test.

In studies of statistical associations of genetically determined phenotypes with disorders, the matter of selection is of importance. The Liverpool bladder cancer patients were people who had survived over a period and who lived in a defined area, so selection factors might have been introduced. It is possible that the survival factor might influence the results in other studies in table 2.

Another point of great importance is the nature of the control populations. Two separate sets of controls were studied by Lower *et al.*⁶ Each separate study has its own control group and the sets of controls were non-random and collected in different ways, one set to accompany the urban Copenhagen

patients and another to accompany the rural Lund patients. In each case the controls were "healthy hospital personnel and hospital patients". A series of geriatric patients without malignant disease constituted the controls of Woodhouse *et al.*⁷ The London-based controls of Cartwright *et al.*⁸ were "healthy subjects from Guy's Hospital", and the Huddersfield controls were either non-bladder cancer urological patients or "patients undergoing cold surgery for various conditions". The six Liverpool control series which were pooled comprised variously healthy medical students, healthy hospital and university staff, and tuberculous patients. It may be speculated that the lack of heterogeneity of the association between the series (table 2) is more convincing in view of the differing nature of the control groups.

Cartwright *et al.*⁸ observed a strikingly high association between the slow acetylator phenotype and bladder cancer in Huddersfield dye workers. No such sub-group of occupational exposure could be defined in the present series where persons had been exposed to a wide variety of industrial chemicals. The association revealed in table 2 is clearly strengthened by the inclusion of the Huddersfield dye workers, but would still be statistically significant ($p < 0.05$) if the data of Cartwright *et al.*⁸ were omitted. Smoking would appear to be a more general environmental association (see table 1 for the data on smoking in the present series).

Table 2 reveals the slow acetylator phenotype to be about 39% more associated with bladder cancer than the rapid acetylator phenotype. Two possible interpretations follow. (1) Slow acetylators are more prone to develop bladder cancer than rapid acetylators. Carcinogenic amines (which occur in tobacco smoke) are known to be polymorphically acetylated. It is suggested, therefore, that the phenotype performing rapid acetylation of these amines may possess a degree of protection against the development of bladder cancer. (2) Slow acetylators have a greater survival with bladder cancer than rapid acetylators.

Obviously prevalence data will not allow either of these interpretations to be eliminated. It will be necessary to acetylator phenotype bladder cancer patients at the time of diagnosis in order to resolve the difficulty. The association shown in table 2 is sufficiently firm to justify such an undertaking.

Addendum

After this article had been submitted Miller (Miller ME. Acetylator phenotype in bladder cancer. *Lancet* 1982; ii: 1348) reported the acetylator phenotypes of 15 bladder cancer patients who had a

positive occupational exposure to bladder carcinogens. Six were slow acetylators. There was no control group.

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